International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Pre- and Postharvest Microbial and Cytogenetic Assessment of Irradiated Philippine Aglibut Sweet Tamarind (AST) Fruit (Tamarindus Indica)

G.E. Santos[a*], J.V. Bagunu[b], D.E. Gracilla[c], E.V. Totaan[d], V.T. Bagunu[e]

[a,b,c,d]*College of Arts and Sciences*

[e]*College of Agriculture Systems and Technology Pampanga State Agricultural University Magalang, Pampanga*

Abstract

The pre-and post-harvest practices of Aglibut Sweet Tamarind farmers were determined and correlated to the microbiological and cytogenetic quality of irradiated and non-irradiated AST fruits (*Tamarindus indica*) using Total Bacterial Count (TBC), Yeast and Mold (YM) test, and C-metaphase chromosome analysis. Some of the practices relative to possible fruit contamination are hand picking, unsanitized container and contact to the soil during harvesting. The initial microbial count is possibly associated with unacceptable farmer's practices during pre-harvest and harvesting process. Microbial analyses established that T3 (irradiated AST fruit at 5kGy) is the best irradiation dose to extend the shelf life of fresh tamarind fruit up to 20 days followed by T2 (irradiated AST fruit at 3kGy) that has also the capability to extend the shelf life up to 15 days. On the other hand, T1 (Irradiated AST fruit at 1 kGy) and control are comparable with each other but cannot prolong the shelf-life of tamarind fruit as compared to T3 (irradiated AST fruit at 5kGy and T2 (irradiated AST fruit at 3kGy). Irradiated AST fruit extract did not cause chromosomal aberrations in white mice in all treatments. Hence, the irradiated Sweet Tamarind Fruit at 5kGy is found to be microbiologically and cytogenetically safe.

Keywords: chromosomal aberration; Cytological profile; clastogenic; Irradiation; Microbial profile.

---

* Corresponding author.

1. Introduction

Post-harvest losses is an emerging problem in Philippine Agriculture which may be due to non-standardized procedure of harvesting, packaging and treatment that lead to substandard quality of agricultural products [1] because of microbial and pest contamination. Fresh fruits such as the Aglibut Sweet Tamarind fruit, one of the flagship commodity of the Pampanga State Agricultural University is not exempted from contamination. Microbial and pest contamination must be reduced to prolong shelf-life of fruits [2].

The use of radiation at the right dosage is considered to be one of the best tools to eliminate pathogenic and spoilage microorganism in food [3] otherwise it may yield to transformation and modification of molecule converting the irradiated product to be toxic [4]. Such intoxication may be due to the formation of hydrogen peroxides and organic peroxides from free radicals altering the genetic material [5], thus, leading to modified molecule, aberrant chromosomes and cancer cells [6].

Hence, this study was conducted to determine the microbial incidence in non-irradiated Aglibut Sweet Tamarind (AST) in association with the AST Farmers' pre- and post-harvest practices, microbial incidence in irradiated Aglibut Sweet Tamarind (AST), chromosome damaging potentials imposed in irradiated Aglibut Sweet Tamarind fruit (Tamarindus rindica) as observed in somatic cells of white mice (Mus musculus L.), and the best irradiation dose that can prolong the shelf life of AST fruits which is microbiologically and cytogenetically safe to consumers. Prolonging the shelf life of the AST fruit will address emerging post-harvest problems of fresh fruits for export such as early food spoilage and shelf-life problems.

1.1 Scope and Delimitation of the Study

The study is delimited to the determination of the microbiologically and cytogenetically safe irradiation dosage for Aglibut Sweet Tamarind Fruit by using cytogenetic C- metaphase scoring, Yeast and mold count and total bacterial count for fungi and bacteria respectively. The experiment was conducted at the Philippine Nuclear Research Institute, Diliman, Quezon City and a survey was undertaken among the Aglibut Sweet Tamarind Farm Workers at the Pampanga State Agricultural University, Magalang, Pampanga.

2. Methodology

2.1 Research Design

This study used both descriptive and experimental method of research. Study 1, in a form of a survey, was done to reveal the pre-and post-harvest practices of AST farmers in Pampanga State Agricultural University, Philippines. The experimental research was done to determine the microbial load of AST using Total Bacterial Count (TBC) and Yeast-and-Mold (YM) Count as well as the possible chromosome breaking potentials of AST when irradiated as a post-harvest measure using Cytogenetic Assessment of C-metaphase chromosomes of the bone marrow cells of white mice.

2.2 Irradiation of AST fruits

The harvested AST fruits packed on a sterile bag (1kg each pack) were submitted to the Philippine Nuclear Research Institute for irradiation treatment. The tamarind fruits were irradiated at 1, 3, 5 kGy gamma radiation. One pack was not irradiated which served as the control for both experiments 1 and 2.

2.3 Microbial Analysis

Ten (10) grams sample of AST fruit (pulp and coat) was used for microbiological analysis as measured by number of colony forming units of bacteria, yeast and molds that were recorded every 5 days for a twenty (20) day observation period. Serial dilution was also applied to determine a reliable count in each of the samples. This was done to determine if the radiation treatment can prolong the shelf-life of the AST fruit based on the irradiated and non-irradiated samples. The time of exposure depends on the computed dose rate needed for the irradiation dose [7].

An improved microbiological testing for TBC (Total Bacterial count, YM (Yeast and Molds) count was adapted from the Laboratory Manual in Food and Water Microbiology] by Cavinta *et al*. [8] of the College of Public Health, University of the Philippine, Manila.

2.4 Cytogenetic analysis

A total of 36 mice were used and distributed to different treatments. Six (6) mice were distributed in each treatment with three replicates per treatment using the Complete Randomized Design. Similar irradiation dose was used for the AST fruit for both Study 2 and 3. An improved cytogenetic technique modified by Gregorio [9] was used to obtain high quality mitotic C- metaphase chromosome spread from bone marrow cells of mice

2.5 Treatment of Data

The data of general and specific chromosomal abnormalities were subjected to One Way Analysis of Variance, and microbial count was subjected in Two Way Analysis of Variance [10]. Tukey's HSD (Honestly Significant Difference) was used to determine if there was significant difference on the means of the different treatments.

3. Results and Discussions

3.1 Pre- and post-harvest practices of AST Farmers

Results of the study showed that 100% of the AST farmers are not using gloves for harvesting. Furthermore, as to the containers used for the harvested fruit, 62.60% used net and 37.50% used sack. Nobody used cloth as a container for harvesting tamarind fruits. All the farmers used hand picking for their method of harvesting while 42.86% used shaking of the branches. No farmer answered they used hook for clipping the fruit. And for the post-harvest practices, all farmers (100%) did sorting of fruits after harvesting and sanitized the baskets prior loading and packing of fruits.

3.2 Microbial Analysis of Non-Irradiated AST fruit

Initial microbiological analysis revealed a count of 1.4 x 10^4 cfu/ml for Total Plate Count and 3.0 x 10^3 cfu/ml for both yeast and mold count. The initial count could be associated with the practices of the farmers during harvest time such as not using gloves (100%), hand picking (100%) and branch shaking (42.86%). Direct hand contact without gloves can contribute to contamination of microorganism on the tamarind fruit. In addition, the farmers' use of branch shaking during harvest can also contribute to contamination because some of the fruits touch the soil. Further, airborne bacteria may also contribute to microbial load in the fruit. Thus, the farmers' wrong practices during harvest could greatly affect the microbial quality of tamarind fruit which lead to combination of innate microbial quality and cross contamination.

3.2.1 Total Bacterial Count in Irradiated AST

T3 (irradiated AST fruit at 5kGy) consistently have the least bacterial activity from Day 0 (mean 1400), Day 5 (mean 0) Day 10 (mean 0) Day 15(0) up to day 20 (mean 26.67) based on the Total Bacterial Count (Figure 1). This was followed by T2 (irradiated AST fruit at 3kGy) with a mean of 1400.00 cu/ml, 12.33 cu/ml, 30 cu/ml and 23333.33 cu/ml in day 0, 5, 10 and 15, respectively. Mean patterns of T1 (irradiated AST fruit at 1kGy) and Control (Non-irradiated AST fruit) is comparable from Day 0 to Day 10. T1 (irradiated AST fruit at 1kGy) and control (Non-irradiated AST fruit) is not comparable on the mean of Day 15 of T2 (irradiated AST fruit at 3kGy) and T3 (irradiated AST fruit at 5kGy). This suggests that T3 (irradiated AST fruit at 5kGy) and T2 (irradiated AST fruit at 3kGy) are the best dosage to reduce total bacterial contamination. Further, Control did fold the microbial count 10 times on Day 10 from Day 0. On the same manner, T1 (irradiated AST fruit at 1kGy) gradually increased the microbial count by 9033.33 cfu/ml to 453733.30 cfu/ml (Figure 1).

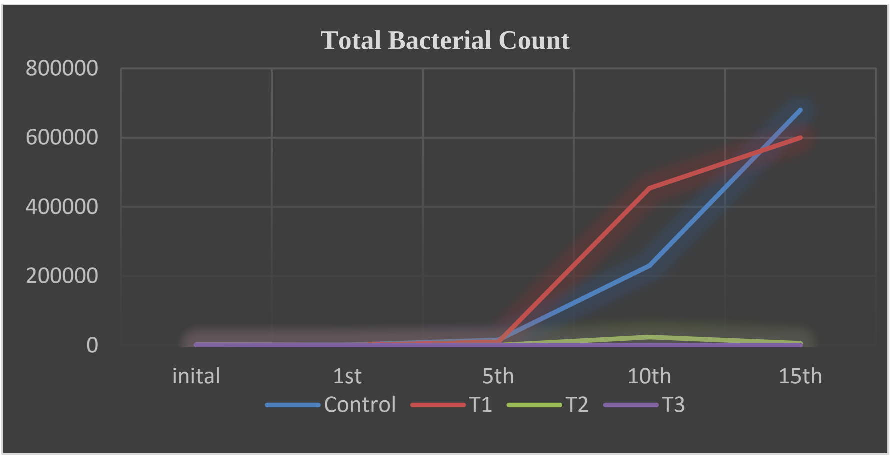


Figure 1: T3-Irradiated Aglibut Sweet Tamarind (AST) at 5Gy exhibited least incidence of bacterial

The results indicate that T3 (irradiated AST fruit at 5kGy) is the best to dosage to delay bacterial proliferation as indicated by the lower Total Plate Count up to 15 days, followed by T2 (irradiated AST fruit at 3kGy). The data in T1 (AST fruit at 1kGy) is comparable to Control (non-irradiated AST fruit) from Day 0 to Day 10 of total Plate Count. On day 10 control group and day 15 T1 (irradiated AST fruit at 1kGy) exceeded the normal standard TPC which is 10^4 (Standard for ready to eat food) [11]. Although, T3 (irradiated AST fruit at 5kGy) and T2 (irradiated AST fruit at 3kGy) are comparable statistically, T3 (irradiated AST fruit at 5kGy) recorded the best dosage for reducing total bacterial count due to the fact that the data from Day 5 -15 are zero at 1:9 dilution. Irradiation in right dosage is known to kill bacteria by damaging the nucleic acid via primary ionization and formation of radicals which give major changes on DNA replication and other physiological function of bacteria [12]. In addition, different dosage of irradiation approved by FDA prolong shelf life of fruit [13] and make the fruits microbiologically safe without affecting the composition of the fruit [14].

3.2.2 Yeast Count in Irradiated AST

In terms of yeast count, T3 (irradiated AST fruit at 5kGy) was shown to have the least microbial activity with the mean of 0 from Day 0 to Day 20, followed by T2 (irradiated AST fruit at 3kGy) showing no significant difference on mean from Day 0 ,(mean 30) Day 5(mean-0), Day 10(mean-0) and Day 15 (mean-101.6667). On Day 20 the mean of yeast on T2 goes up to 6000.00 which are significantly different to Day 0-Day 15 of T2 (irradiated AST fruit at 3kGy). The Control (Non-irradiated AST fruit) and T1 (irradiated AST fruit at 1kGy) recorded an acceptable microbial quality up to Day 10 and Day 5 respectively (Figure 2).

contamination

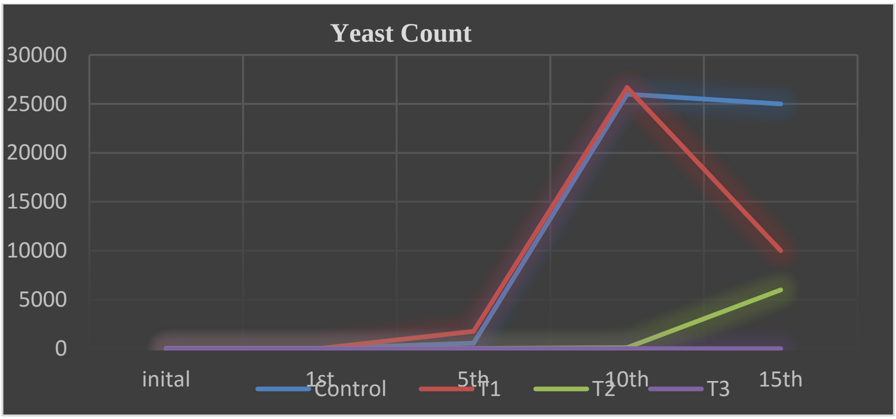


Figure 2: T3-Irradiated Aglibut Sweet Tamarind fruit (AST) at 5Gy exhibited the least incidence of yeast contamination

Prolonging the shelf life of tamarind based on yeast count T3 (irradiated AST fruit at 5kGy) shows to be effective from Day 0 to Day 20. This was followed by T2 which is microbiologically acceptable up to Day 15. T1 and control are both microbiologically unacceptable on Day 10, because they exceeded the normal yeast count which is $<10^3$cfu/ml (Ministry of Health, 1995). T3 (irradiated AST fruit at 5kGy) therefore, is the best dose, to stop the proliferation of yeast up to Day 20. T2 can also be considered as good dose, but T3 will be safer to use. Yeast reproduction is altered and stopped by radiation in correct dosage. In the same mechanism with bacteria, radiation kills yeast cells via radical formation and also by primary ionization which result with DNA system damage [12].

3.2.3 Mold Count in Irradiated AST

The mold count of T3 (irradiated AST fruit at 5kGy) has the least microbial activity with a mold count mean of 30, 0, 0, 0 and 4000 from Day 0 to Day 20 respectively, Thus T3 (irradiated AST fruit at 5kGy) can extend the shelf life of tamarind up to 15 days which is 3 folds of the normal shelf life. Second to T3 (irradiated AST fruit at 5kGy) is T2 (irradiated AST fruit at 3kGy) with a mold count from Day 0(mean 30), Day 5 (mean=4.33) Day 10 (mean=20) Day 15 (mean=400). T2 (Irradiated AST fruit at 3kGy) can extend the shelf life 3 folds of the normal shelf life which is 5 days according to Reyes 2014, T1 (irradiated AST fruit at 1kGy) and control (Non-irradiated AST fruit) group are microbiologically acceptable up to 10th Day only because the mold count exceeded the acceptable limit for mold count which is $<10^3$ (Ministry of Health, 1995). Thus, this suggests that T3 is the best dosage in reducing and controlling the proliferation of molds. Mold growth can also be controlled and inhibited by ionizing radiation. Inhibition of mold growth in fruit may lead to long shelf life of fruit [13] (Figure 3).

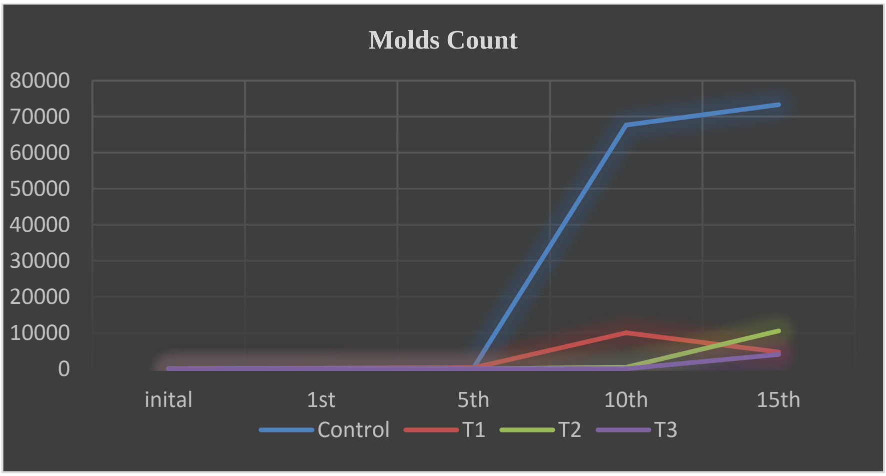
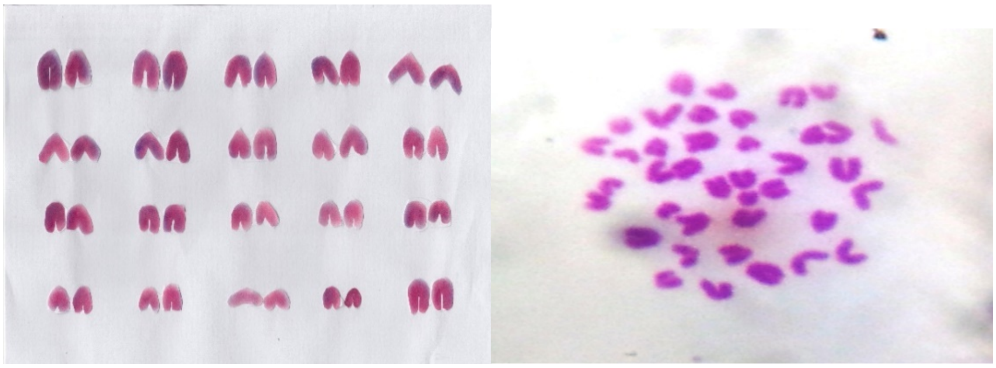


Figure 3: T3 (Irradiated Aglibut Sweet Tamarind (AST)) fruit at 5Gy exhibited the least incidence of Molds Count

3.3 Cytogenetic study of Irradiated AST fruit on the bone marrow cells of White Mice

The results indicated that there was no effect of the irradiated AST Fruit Extract on the chromosome structure of the treated mice (Figure 4). Low irradiation dosage cannot form carcinogenic cyclic compound which can intercalate the genetic material [13] that results to chromosomal aberration. As reported, 10kGy dosage for some studied fruit did not become genotoxic or radioactive [12]. This may be attributed to the presence of minerals, potassium, phosphorous, calcium and magnesium found on the Tamarind fruits [15] which are rich sources of anti-oxidant that can reduce the effects of radicals formed by ionizing radiation. These anti-oxidants impose scavenging effects on the free radicals produced by Ionizing radiation [16].



a b

Figure 4: Normal Chromosome spread (a) and karyotype (b) of irradiated AST fruit.

4. Conclusion and Recommendation

4.1. Aglibut Sweet Tamarind (AST) fruit irradiated at 5kGy gamma radiation is microbiologically and toxicologically safe up to 15 days of treatment as reflected on the mean microbiological count and chromosomes structures, respectively, hence, recommended for use in the tamarind fruit industry through export, high post-harvest yield with a promise of high quality, toxicologically and microbiologically safe Aglibut Sweet Tamarind fruit.

Acknowledgment

Acknowledgment is due to the staff of the Philippine Nuclear Research Institute for the training of research staff and use of their radiation facility and the Pampanga State Agricultural University Sweet Tamarind Research Center for the AST samples used in the study.

References

[1] D. S Murthy. and T. M. Ganjanah. Marketing and Post-Harvest in Fruits. Availability and Implication. Indian Journal of Agricultural Business. 16:24. 2009.

[2] Berger, et al. Gamma Radiation against toxigenic fungi in food, medical and aromatic herbs. Science Against Microbial Pathogens. 4:29-35.2010.

[3] S. Aquino, S. Gamma Radiation against toxigenic fungi in food, medical and aromatic herbs. Science Against Microbial Pathogens. 6:2. 2011.

[4] D. Endoh, D. Micronucleus test in mice fed on an Irradiated Diet. Jph. Jvet. Res., 37:41-47. 1989.

[5] J. Schubert. Mutagenicity and Cytotoxicity of Irradiated Food and Component. Bul. Org Mon, Sante. 873:94. 1969.

[6] D.E. Gracilla, and J.V. Bagunu. Radiation Cytogenetic studies on Philippine Ashitaba (Gynura nepalensis D.C.).International Journal of Sciences: Basic and Applied Research. 17:1. 2014.

[7] L.L. Solomon, Personal Communication, December 19, 2014- 6pm, Nuclear Services Division. Philippine Nuclear Research Institute, Commonwealth Avenue, Diliman, Quezon City Philippines.

[8 L.L Cavinta,., et al. Laboratory Manual in Food and Water Microbiology: Department of Medical Microbiology, College of Public Health University of the Philippines Manila. 2009.

[9] F.S. Gregorio,. Experiment No.2 Experimental Procedure and Theory in Cytogenetic Laboratory. Philippine Nuclear Research Institute. 2009.

[10]. Statsoft Inc. (1999). Statistica 99th Edition. USA. www.stat.knowledge.com

[11] Ministry of Health (1995). Microbiological Reference Criteria for Food, Food Administration section of the Ministry of Health. Institute of Environmental Science and Research.

[12] B. Kalyani, B. and K. Manjula, K. Food Irradiation - Technology and Application, International Journal of Current Microbiology and Applied Sciences ISSN: 2319-7706,3:4:549-555(2014

[13] IAEA (International Atomic Energy Agency), (2009). Irradiation to ensure the safety and quality of prepared meals, Vienna, Austria,. 375.

[14] J. Farkas,. Charged particle and photon interactions with matter, In: Mozumder, A. & Hatano, Y. (eds): Food Irradiation, Marcel Dekker, New York, 785–812. 2004.

[15] K. Tomlins, (2008). Food safety and quality management. Food Africa. http://foodafrica.nri.org/safety/safetydiscussions1.html

[16] BAIF . Fruits for the future: Tamarind messages programme and technologies on sustainability Newsletter, www.baif.com/mpts6.htm.p.25. 2002.

[17] S.S. Bhattacharya, and R. K. Mukherjee.. Functional and nutritional properties of (Tamarindus indica) kernel protein. Food chemistry, 49: 1-9. 1994.